

LBNL 201 (10412981)

IN THE SPECIFICATION

Please amend the specification as follows:

[0058] Nonhuman antibodies are highly immunogenic in human thus limiting their therapeutic potential. In order to reduce their immunogenicity, nonhuman antibodies need to be humanized for therapeutic application. Through the years, many researchers have developed different strategies to humanize the nonhuman antibodies. One such example is using HuMAb Mouse® ~~“HuMAb Mouse”~~ HuMAb Mouse® technology available from MEDAREX, Inc. (Princeton, NJ). ~~“HuMAb Mouse”~~ is a strain of transgenic mice that harbors the entire human immunoglobulin (Ig) loci and thus can be used to produce fully human monoclonal pT2609 and pS2056 antibodies.

[079] 20 bp oligomer primers were designed and ordered from Operon (Alameda, CA) using SEQ ID NO: 4 (the nucleotide sequence of DNA-PKcs, GenBank Accession Number: P78527) to create primers to amplify cDNA sequence that encodes the phosphorylation sites, T2609 and S2056. Designed DNA-PKcs cDNA fragments that cover the phosphorylation sites in DNA-PKcs found by mass spectrometry were PCR amplified from the full-length DNA-PKcs cDNA (isolated and described by several of the inventors in Kurimasa et al., *Mol Cell Biol* 19: 3877-3884, 1999) using the custom designed PCR primers under normal PCR thermal cycling conditions. The reactions were carried out using *pfu* DNA polymerase (Stratagene, La Jolla, CA) and GeneAmp® ~~GeneAmp~~ 9600 thermocycler (Perkin Elmer). The amplified cDNA fragments were cloned in frame into GEX-KG vector (Guan & Dixon 1991 *Analytical Biochem* 192:262-67) for fusion between domains of DNA-PKcs and GST.

[096] First, a 3kb Hind III fragment of DNA-PKcs cDNA covering T2609 was used as the template for generating the T2609A mutation of DNA-PKcs cDNA. Site-directed mutagenesis was performed using the QuickChange® ~~Quick Change~~ site-directed mutagenesis kit (Stratagene, La Jolla, CA) and the forward (tccgatgtttgtggaggaccaggcctcccagggc) (SEQ ID NO: 27) and reverse (gccctgggaggcctggtcctccacaaacatcgga) (SEQ ID NO: 28) primers. The mutated DNA-PKcs cDNA fragment was assembled back into the full length DNA-PKcs cDNA as described in Kurimasa et al., *Mol Cell Biol* 19: 3877-3884, 1999. Cells

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were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air by using alpha-MEM medium supplemented with 10% fetal calf serum, 100 U of penicillin per ml, and 100 µg of streptomycin per ml. Transfection of the DNA-PKcs expression plasmid was performed with a calcium phosphate transfection system (Catalog no. 18306-019; Gibco-BRL, Gaithersburg, MD). For each 10⁶ cells in a 100-mm tissue culture dish, 10 µg of the DNA-PKcs expression vector and 10 µg of the pSV2neo or pPur plasmid were transfected.

[108] The phosphopeptides were conjugated to KLH by cross-linker Sulfo-SMCC (Pierce Biotechnology, Inc., Rockford, IL) which forms a disulfate bridge with the cysteine residues placed at the C' terminal of the synthesized peptides and cysteine residues on KLH. The mixture was then incubated with rotation at RT for 2 hours to block unreacted SMCC. Dialysis against 2L PBS was done with at least 2 buffer changes. Dialysis may proceed overnight. In place of dialysis, a Sephadex® ~~SEPHADEX~~ G-25 column (Amersham Biosciences, Piscataway, NJ) may be used again to desalting.

[111] The phosphospecific antibodies were affinity purified through a phosphopeptide-conjugated Sepharose® ~~Sepharose~~ CL-4B column. SEQ ID NO: 1 was made as an unphosphorylated peptide, N'-PMFVETQASQGTC-C' which corresponds to the T2609 site unphosphorylated. SEQ ID NO: 2 was made as an unphosphorylated peptide, N'-QSYSSQDPRPAC-C', to correspond to the S2056 site unphosphorylated.